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Factor IX Concentrate: Preparation by a Simple Method and Clinical Use

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The principal application of Factor IX concentrates is for hemophilia B patients threatened with circulatory overloading when severe hemorrhages or surgical treatment make it necessary to replace the missing coagulation factor by whole blood or plasma transfusion.

Unlike the preparation of a Factor VIII concentrate, which can be done very simply and efficiently with the cryoprecipitation procedure according to Pool (1), the purification of Factor IX for clinical use is rather complicated. With plasma as starting material, the result of the usual methods is not only the concentration of Factor IX on a protein basis, but also, due to their similar adsorption properties, the purification of the other factors of the so-called prothrombin complex. Consequently, this type of preparation is useful not only for patients with hemophilia B, but also for those with a deficiency of the complex of Factors II, VII, IX and X, such as occur in severe liver function disturbances, real vitamin K deficiency, and, last but not least, the relative vitamin K deficiency induced by treatment with coumarin.

In situations where there is no time to wait for the effect of vitamin K administration*, such as intracranial hemorrhage during

* Intravenously administered vitamin K_1 takes a minimum of 8 hr to provide a safe level of the prothrombin complex.

anticoagulant treatment (2), or when hemorrhagic complications must be prevented or treated, as in cases of severe disturbances of liver function, the administration of purified Factors II, VII, IX and X may be indicated. Lastly, severely disturbed hemostasis, seen in congenital deficiencies of Factors II, VII or X individually, may also be an indication. In practice, however, the main use of the concentrate is for the substitution treatment in hemophilia B to combat serious hemorrhagic complications, which is the main subject of this report.

In Leiden, during the period between 1960 and 1967, eight patients suffering from hemophilia B (three severe, one moderate, and four mild) were given substitution therapy with the French preparation P.P.S.B. (Prothrombin-Proconvertin-Stuart factor-antihemophilic factor B), obtained from the Centre National de Transfusion Sanguine in Paris (3, 4, 5). For the evaluation of the therapeutic results of such treatment, a good method for the determination of Factor IX is indispensable. Up to 1964, we used a one-stage method having a standard deviation of at least 10% of the values obtained. By modification of the one-stage method according to Hardisty and Macpherson (6) and the use of a semiautomatic reading of the coagulation times, Veltkamp (7) was able to reduce the coefficient of variation to 6%-7%. Using this reliable method of determination, we obtained values for the activity of P.P.S.B. *in vitro* which showed good agreement with those given by the manufacturer. The administration of P.P.S.B. to patients showed that the *in vivo* characteristics of this purified Factor IX agreed closely with those of Factor IX in normal plasma. The biological half-life amounted to approximately 20 hr. With an equilibration half-life of several minutes, half of the amount had disappeared from the intravascular space. In patients retaining some capacity to produce Factor IX (the mild and moderate types), this production was not suppressed by the substitution treatment.

These data made it possible to formulate a general dosage scheme for the daily required quantities of Factor IX, expressed as the equivalent of the activity of H ml net plasma (i.e., ACD excluded) and administered as a constant drip infusion, as follows:

$$H = 17 \times P \times L$$

in which H = net plasma equivalent in ml; P = plasma volume in liters; L = desired rise of the blood level in per cent; the constant 17 is derived from $\frac{0.693 \times 24 \times 2 \times 1000}{20 \times 100}$, in which $0.693 = \ln 2$; 24 = duration of the infusion in hours; 2 = multiplication factor for

calculation of the actual distribution space of Factor IX, and 20 = biological half-life of Factor IX in hours.

The dosage for patients of the mild or moderate types is calculated by subtracting the pretransfusion value from the desired level.

Table 1 shows the desired Factor IX concentration in the case of severe hemorrhage or surgical treatment, as well as the quantity of plasma required for an arbitrarily chosen hemophilia B patient. Fig. 1 gives an example of substitution treatment with the French

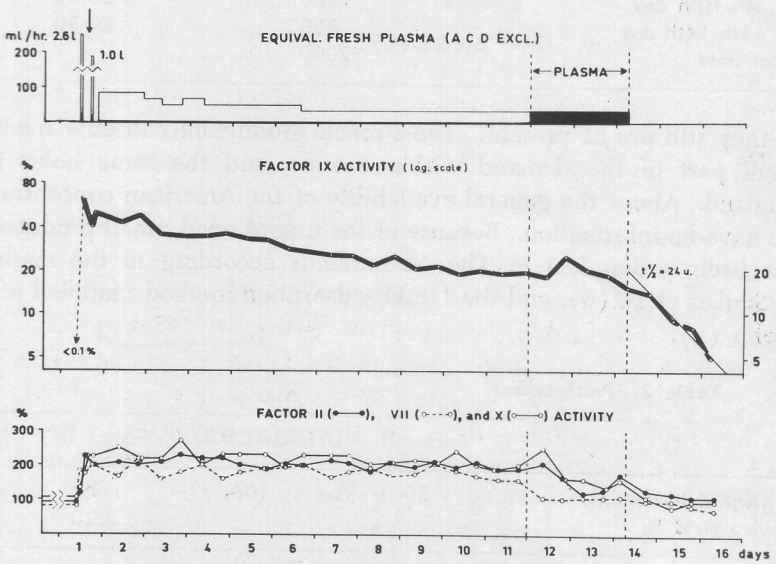

Fig. 1. Data obtained during substitution therapy; patient I 125/66; 19-year-old male; Factor IX < 1% of normal; operation for talipes equinus.

P.P.S.B. in a patient suffering from severe hemophilia B. After a spontaneous intramuscular hemorrhage in the calf of his left leg, a talipes equinus developed for which the boy was treated surgically under P.P.S.B. protection in 1966. The postoperative course was uneventful.

In the other seven cases P.P.S.B. gave excellent results. The hemostatic effect was completely satisfactory. It is very important to keep in mind that in clinical conditions in which the turnover rate of Factor IX is increased, as in cases with a general acceleration of catabolism (e.g., fever, hyperthyroidism, and anemia), the requirement can rise to three times the normal value (4).

The theoretical data have little practical value as long as concentrated Factor IX preparations remain as scarce and difficult to obtain

Table 1. Dosage Scheme for Adult Hemophilia B Patient (Factor IX — 0% of Normal, Body Weight 70 kg, Plasma Volume 2.5 liters)

	ml Net Plasma	In vivo Concentration Obtained %
Loading dose	2000	40-50
Daily dose, (constant drip infusion)		
1st-4th day	1700	40-50
5th-10th day	1275	30-40
11th-14th day	850	20-30
Total dose	2000	

as they still are at present. The French production can only meet a small part of the demand within France, and the same holds for England. About the general availability of the American concentrates we have no information. Because of the urgent need, small production has been undertaken in The Netherlands according to the method of Soulier et al. (8), and the DEAE-adsorption method modified after Melin (9).

Table 2. Prothrombal

	Factor II	Factors VII/X	Factor IX
In vitro yield %	50	106	72
In vivo yield %			±20

In general, it is impossible for the smaller laboratories to adopt the method of Soulier et al., because this procedure calls for the use of EDTA blood, and packed cells cannot be used without a special preliminary treatment. Furthermore, the method is technically rather complicated and laborious. The latter holds even more strongly for the method of Bidwell et al. (10). As a consequence, there is a great deal of interest in a procedure for the purification of Factor IX from ordinary blood bank material, i.e., citrated blood. This would leave all the packed cells for clinical use without extra processing and also permit the production of cryoprecipitate and albumin and gamma globulin preparations.

In our laboratory we have solved this problem in the following way: if the supernatant citrated plasma remaining after cryoprecipitation according to Pool is used, the prothrombin complex cannot be adsorbed with calcium phosphate or barium sulfate. But with a well-

chosen aluminum hydroxide preparation, this complex can be removed virtually completely from the plasma, leaving the albumin and gamma globulins in the plasma for further isolation.

Purification of Factors II, VII, IX and X of 5 to 15 times, based on the protein content, is adequate for the clinician's purposes. Thus a higher yield for coagulation activity and a simpler preparation method are possible, compared to concentrates more highly purified.

After adsorption we elute directly with a phosphate solution

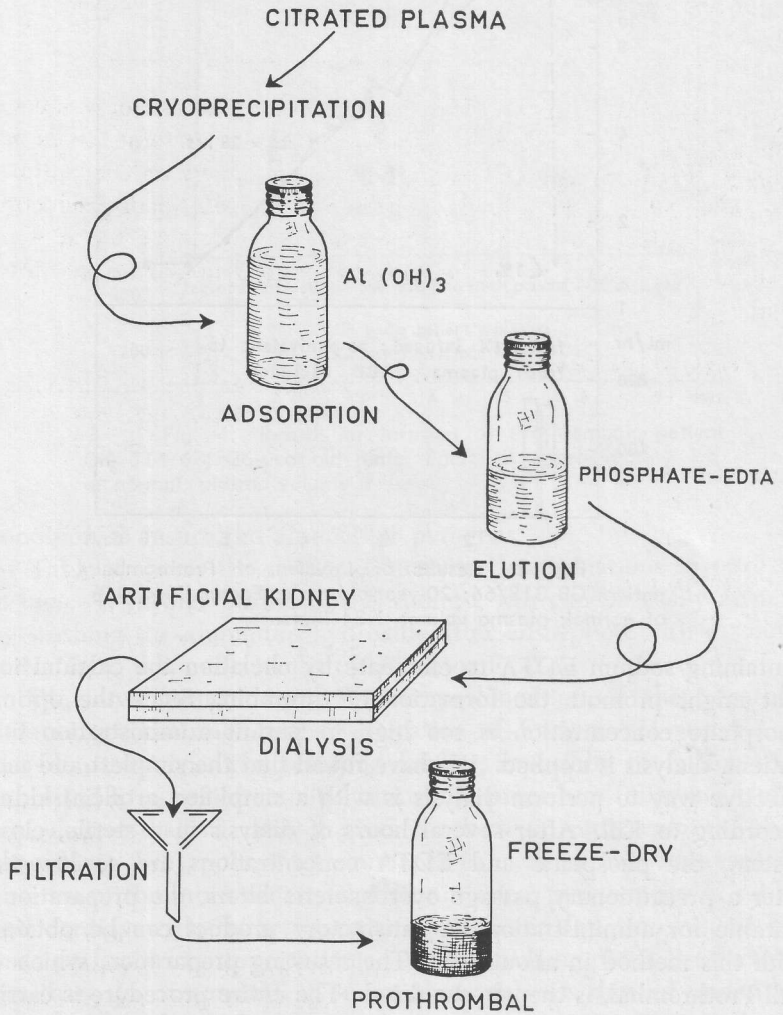

Fig. 2. Diagram of procedure of preparation of Prothrombal.

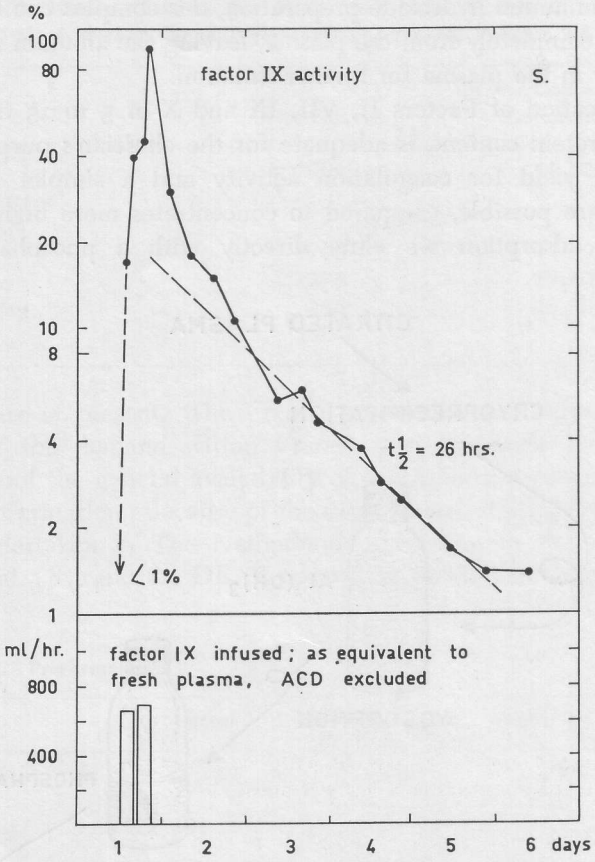

Fig. 3. Results of infusion of Prothrombal; patient GB 318/66; 20-year-old male; Factor IX < 1% of normal; plasma volume 1.84 liters.

containing sodium EDTA to eliminate by chelation the calcium ions that might promote the formation of thrombin. Since the optimal phosphate concentration is too high to permit administration to a patient, dialysis is applied. We have found that the simplest and most effective way to perform dialysis is with a simplified artificial kidney according to Kiil. After several hours of dialysis in a sterile, closed system, the phosphate and EDTA concentrations are so low that after a precautionary passage over bacteria filters, the preparation is suitable for administration. A satisfactory product can be obtained with this method in about 8 hr. The resulting preparation, which we call Prothrombal, is then freeze-dried. The entire procedure is carried out under aseptic conditions, which we consider to be an important

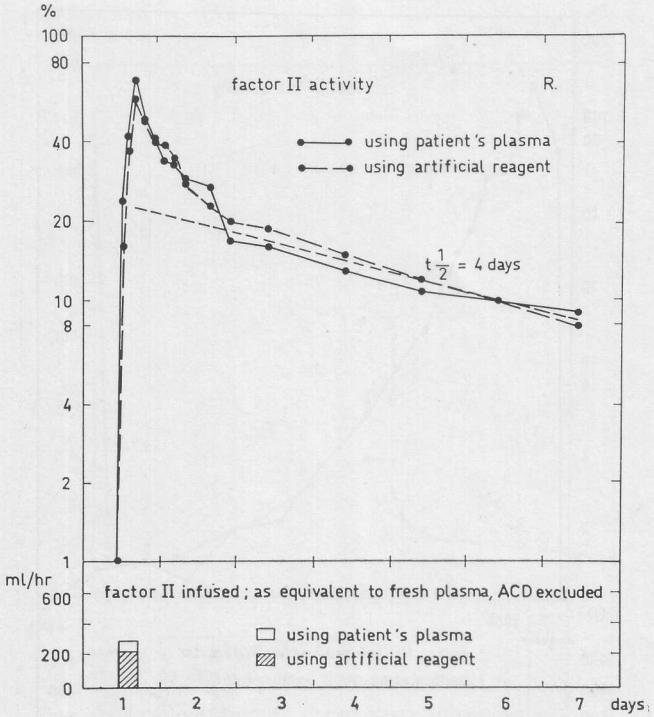

Fig. 4. Results of infusion of Prothrombal; patient GK 314/68; 26-year-old male; Factor II approximately 1% of normal; plasma volume 3 liters.

condition to insure the absence of pyrogens.

Table 2 shows the results of activity determinations *in vitro* and *in vivo*. If further purification is desired, this can be easily achieved by washing the aluminum hydroxide after adsorption with a solution of NaCl or, more effectively, of citrate or EDTA.

We have found, however, that prolongation of the adsorption time causes not only further activation of Factors VII and IX, but also denaturation. The resulting lability, especially of Factor IX, is expressed in a loss of activity during the procedure following elution, e.g., during dialysis. Fig. 2 gives a schematic representation of the procedure. We apply strict standards to each batch with respect to absence of thrombin activity, toxicity in mice, pyrogenicity in rabbits, and cardiovascular effects in cats or dogs. So far, transfusions have been performed in three patients with severe hemophilia B (Factor IX less than 1% of normal). None of these patients showed undesirable

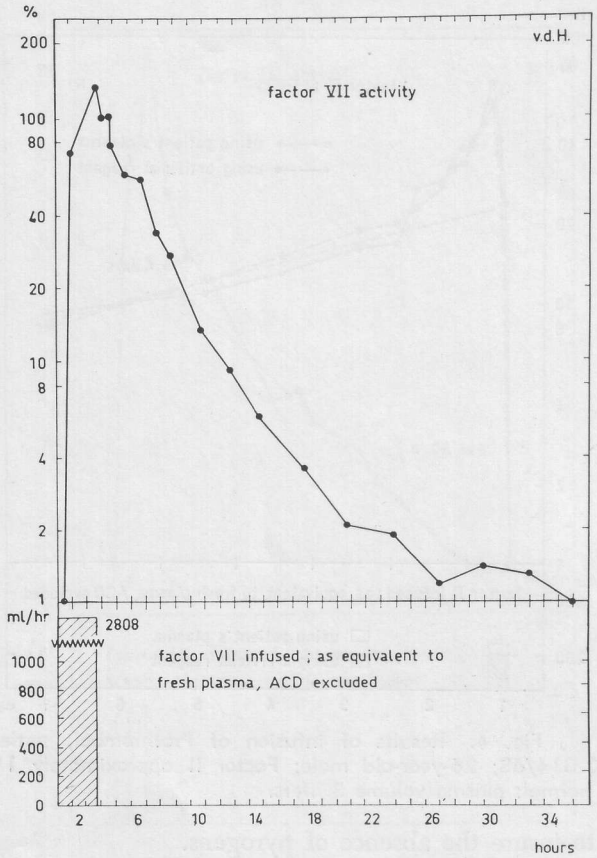

Fig. 5. Results of infusion of Prothrombal; patient GK 347/68; 20-year-old female; Factor VII < 1%; plasma volume 1.9 liters.

reactions. Fig. 3 gives the data for one of these cases as illustration, showing that an activity of almost 100% can be reached *in vivo* without difficulty. Calculations based on the results of the three hemophilia B patients showed that the *in vivo* yield of Factor IX was one-fourth of the *in vitro* activity of the preparation administered. The divergence is probably related to the observation that Factor IX is activated during adsorption, which results in an increased lability expressed in the rapid initial drop of the level of this factor. The subsequent rate of disappearance agrees well with the value found for other transfusions ($T_{1/2}$ approximately 20 hr). The final yield *in vivo* is in approximate agreement with the Factor IX yield obtained with the French method (15% to 20%).

The results of a transfusion with our preparation in a case with a

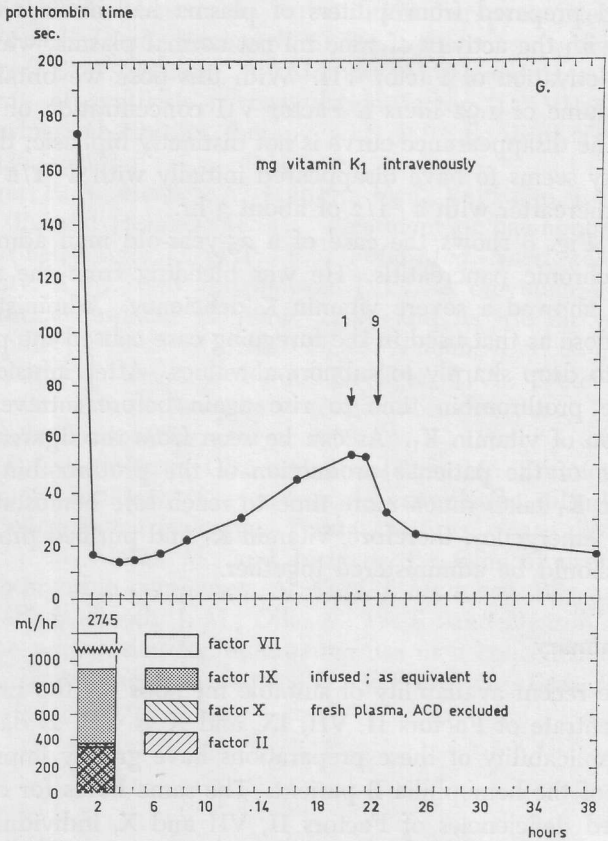

Fig. 6. Results of infusion of Prothrombal; patient GBOE 1175/68; 25-year-old male; plasma volume about 2.5 liters; severe vitamin K deficiency.

congenital prothrombin deficiency are shown in Fig. 4. This patient has a hemorrhagic diathesis entirely comparable to that of a severe hemophiliac and resulting, among other things, in spontaneous bleeding in joints and muscles. The total quantity of prothrombin administered corresponds to the activity of about 1.5 liters net plasma. The Factor II concentration reached in this patient agrees rather well with his plasma volume of 3 liters. After a rather slow equilibration during the first day, the disappearance rate of Factor II corresponded to the biological half-life of about four days.

Fig. 5 shows the effect of the administration of our preparation to a girl with congenital Factor VII deficiency who suffered from severe hemarthroses and menorrhagia. The administered dose of

Factor VII prepared from 4 liters of plasma had an *in vitro* value agreeing with the activity of 7800 ml net normal plasma, which again indicates activation of Factor VII. With this dose we obtained in a plasma volume of 1.92 liters a Factor VII concentration of 132% of normal. The disappearance curve is not distinctly biphasic; the Factor VII activity seems to have disappeared initially with a $T_{1/2}$ of about 2 hr and thereafter with a $T_{1/2}$ of about 3 hr.

Lastly, Fig. 6 shows the case of a 25-year-old man admitted because of chronic pancreatitis. He was bleeding from the nose and gums and showed a severe vitamin K deficiency. Administration of the same dose as that used in the foregoing case caused the prothrombin time to drop sharply to subnormal values. After infusion we allowed the prothrombin time to rise again before intravenous administration of vitamin K_1. As can be seen from the figure, maximal stimulation of the patient's production of the prothrombin complex by vitamin K_1 takes much more time to reach safe hemostatic levels. In case of emergency, therefore, vitamin K_1 and purified prothrombin complex should be administered together.

Summary

The recent availability of suitable methods for the preparation of a concentrate of Factors II, VII, IX, and X as well as data on the clinical applicability of these preparations have greatly improved the prognosis of the hemophilia B patient. The same holds for congenital or acquired deficiencies of Factors II, VII and X, individually or in combination. This concentrate therefore also represents an immediately effective antidote for coumarin congeners. A simple method for the preparation of a Factor IX concentrate is described. Packed cells, thrombocyte suspensions, cryoprecipitate, albumin and gamma globulins can be prepared without any loss or difficulty from the same citrated blood.

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